Pestic. Sci. 1997, **50**, 104–110

Quantitative Structure–Activity Relationships of DDT-Type Compounds in a Sodium Tail-Current in Crayfish Giant Axons

Keiichiro Nishimura* & Hiroshi Okimoto

Research Institute for Advanced Science and Technology, Osaka Prefecture University, Sakai, Osaka 593, Japan

(Received 20 May 1996; revised version received 4 October 1996; accepted 18 February 1997)

Abstract: Effects of DDT-type compounds including 1,1-bis(*para*-substituted phenyl)-2,2-dichlorocyclopropanes (DCC-series compounds) on sodium currents in crayfish giant axons were measured under voltage-clamp conditions. Variations in the activity to prolong the tail-current that was observed upon step repolarization of the membrane were quantitatively analysed by use of physicochemical parameters of aromatic substituents and regression analysis. Introduction of lengthy and narrow substituents was favourable to the activity. Variations in the activity were parabolically related to the hydrophobicity, optimum value being around that of H. DDT- and prolan-series compounds were 2–3 times more active than DCC-series compounds when other structural factors were the same. Insecticidal activity of the compounds was linearly correlated with the tail-current activity when the hydrophobic factor was separately considered. The insecticidal activity of DDT-series compounds was 2·5 times higher than that of others when the other factors were the same.

Key words: DDT-type compounds, bis(*para*-substituted phenyl)dichlorocyclopropanes; sodium tail-current, voltage clamp method, crayfish giant axon, QSAR

1 INTRODUCTION

The insecticidal activity of DDT and related compounds is due to their neurotoxic effects, including induction of repetitive discharges, elevation of depolarizing afterpotential, and modification of membrane currents.[1–3] Among the membrane currents, the effects inducing a long-lasting sodium tail-current seem to be the most important.[1–5]

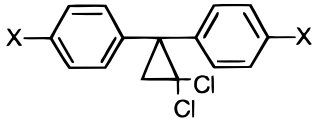
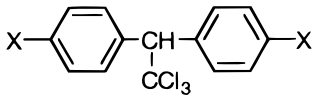
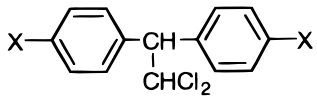
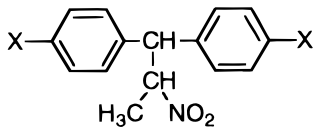
Previously, we measured the activity of a set of DDT and related compounds in inducing repetitive discharges in the central nerve cord of the American cockroach (*Periplaneta americana* L) and quantitatively analysed substituent effects on the activity using the aromatic substituent parameters.[6,7] We found that the most important factor affecting the activity was a steric property represented by the volume. Since the insecticidal activity of these compounds against the American cockroach was correlated well with the repetitive activity, after separating the hydrophobic factor, we considered that the nerve effect is the principal effect responsible for insecticidal activity.

To extend our study, we evaluated the nerve activity of a set of DDT and related compounds including 1,1-bis(*para*-substituted phenyl)-2,2-dichlorocyclopropanes to prolong the decay of the sodium tail-current, which was measured in crayfish giant axons under voltage-clamp conditions by the double sucrose-gap technique. The activity values were quantitatively analysed using physicochemical substituent parameters and a regression method. We also analysed the relationship of the nerve activity with the previously measured insecticidal

* To whom correspondence should be addressed.

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

TABLE 1
Biological Activities and Physicochemical Parameters of DDT-Type Compounds

		log τ		*log(1/MLD)*[a]					
No.	*X*	*Obsd*[b]	*Calcd*[c]	*Obsd*	*Calcd*[d]	ΔL	ΔB_5	π	*log P*
									
	(DCC series)								
1	H	0·11 (1)	0·20	6·49	6·60	0·00	0·00	0·00	4·88
2	F	0·67 (2)	0·72	7·14	7·00	0·59	0·35	0·14	5·16
3	Cl	1·34 (2)	1·28	7·04	7·38	1·46	0·80	0·71	6·30
4	Br	1·57 (2)	1·43	7·41	7·51	1·76	0·95	0·86	6·60
5	Me	1·07 (2)	0·67	6·76	7·21	0·81	1·04	0·56	6·00
6	*i*-Pr	0·61 (1)	0·50	6·48	6·58	2·05	2·17	1·53	7·94
7	*t*-Bu	−0·23 (1)	−0·06	<8·40	5·78	2·05	2·17	1·98	8·84
8	OMe	1·07 (2)	1·29	7·40	7·34	1·92	2·07	−0·02	4·84
9	OEt	1·74 (1)	1·79	7·61	7·77	2·74	2·36	0·38	5·64
10	OPr(*n*)	1·45 (5)	1·61	7·64	7·37	3·99	3·42	1·05	6·98
11	OPr(*i*)	1·01 (3)	0·92	7·32	7·09	2·74	3·10	0·85	6·58
12	OBu(*n*)	1·49 (3)	1·46	7·10	7·24	4·80	3·79	1·55	7·98
13	CH_2Br	0·82 (1)	0·63	<7·72	6·96	2·03	2·75	0·79	6·46
14	SMe	1·21 (3)	1·32	7·63	7·30	2·24	2·26	0·61	6·10
15	SEt	1·43 (1)	1·23	7·58	7·35	3·10	2·97	1·07	7·02
16	NO_2	0·82 (2)	1·12	7·00	7·20	1·38	1·44	−0·28	4·32
17	SO_2Me	0·32 (1)	0·39	<7·15	6·98	2·05	2·17	−1·63	1·62
									
	(DDT series)								
18	Cl	2·01 (1)	1·60	8·13	8·30	1·46	0·80	0·71	6·29
19	OMe	1·43 (3)	1·61	8·17	8·03	1·92	2·07	−0·02	4·83
20	OEt	1·88 (1)	2·11	8·31	8·28	2·74	2·36	0·38	5·63
									
	(DDD series)								
21	Cl	1·09 (1)	1·28	7·30	7·23	1·46	0·80	0·71	5·93
22	OMe	1·24 (1)	1·29	7·40	7·51	1·92	2·07	−0·02	4·47
23	OEt	2·00 (1)	1·79	8·10	8·01	2·74	2·36	0·38	5·27
									
	(Prolan series)								
24	Cl	1·67 (1)	1·78	8·19	7·75	1·46	0·80	0·71	5·33
25	OMe	1·68 (1)	1·79	8·00	7·89	1·92	2·07	−0·02	3·87
26	OEt	2·50 (1)	2·29	8·19	8·45	2·74	2·36	0·38	4·67

[a] MLD = minimum lethal dose (mol per insect).
[b] The figures in parentheses show the number of axons used.
[c] From eqn (6).
[d] From eqn (9).

activity. These findings were compared with those obtained previously for the repetitive activity.

2 MATERIALS AND METHODS

2.1 Compounds

Compounds listed in Table 1 were the same as those used previously.[6,7] Among them, compounds **1, 2, 4, 5, 9, 13, 14** and **16** were generous gifts of Dr G. Holan at CSIRO, Australia. In this study, compounds **1–17, 18–20, 21–23** and **24–26** were called DCC-, DDT-, DDD- and prolan-series compounds, respectively, in short. The test compounds were dissolved in methanol (MeOH) for the insecticidal test and in dimethyl sulfoxide (DMSO) for the nerve test. Commercially obtained piperonyl butoxide (PB), which was used as an inhibitor of oxidative metabolism, was dissolved in methanol.

2.2 Measurement of the sodium current under voltage-clamp conditions

Voltage-clamp experiments were performed with giant axons of crayfishes, *Procambarus clarkii* (Girard), by the double sucrose-gap technique according to the procedure described.[8,9] The sodium current was measured after eliminating the potassium ions from the reported standard internal solution by substituting with cesium ions.[8] The holding membrane potential was -100 mV. The ohmic leakage and capacitive currents associated with step changes in the membrane potential were estimated by extrapolation of the currents associated with step hyperpolarization to -140, -130 and -120 mV and were subtracted from the current observed. Test solutions were prepared by addition of a small portion of the DMSO solution of test compounds into the potassium-free internal saline, and were applied internally to the axon. Most of solutions were turbid because of their poor solubility, but were used as such. In these cases, the concentration of compounds was taken to be that for complete solution. The final concentration of DMSO in the internal saline was lower than 5 ml litre^{-1}. DMSO alone at this concentration had no effect on the sodium currents. Measurements were carried out at 6($\pm$2)°C.

2.3 Measurement of insecticidal activity against American cockroaches

The insecticidal activity of the compounds was measured against male adult American cockroaches, *Periplaneta americana*, by a procedure described previously.[7] Unless otherwise noted, a methanol solution (1 μl) containing PB (50 μg) was first injected into the abdomen of the insect. The insecticidal activity values, log(1/MLD), where MLD was defined as the minimum lethal dose (MLD, mol per insect) listed in Table 1 were taken from our previous paper.[7] The standard error of the log(1/MLD) value was within $\pm$0·1.

2.4 Correlation analysis

Variations in the neurophysiological activity, log τ, which will be defined below, were analysed with physicochemical parameters of aromatic substituents X and regression analysis by eqn (1).[10,11]

$$\log \tau = a\Delta S - b(\Delta S)^2 + c\pi - d\pi^2 + \text{constant} \quad (1)$$

The ΔS is the STERIMOL maximum length (ΔL) or width (ΔB_5) of substituents relative to that of H.[12] π is the hydrophobic substituent parameter derived from log P of monosubstituted benzenes, where P is the partition coefficient in the 1-octanol/water partitioning system.[13] a, b, c, d and the constant were calculated by regression analysis. $(\Delta S)^2$ and π^2 were added to identify the optimum values, so that b and $d \geq 0$. Table 1 lists the substituent parameters.

We examined the relationship between insecticidal and neurophysiological activities by eqn (2).

$$\log(1/\text{MLD}) = a(\log \tau) + b(\log \text{P}) - c(\log \text{P})^2 + \text{constant} \quad (2)$$

Log P values that represent the hydrophobicity of the molecule were cited from our previous paper.[7] The $(\log \text{P})^2$ term was added to identify the optimum hydrophobic effect, so that $c \geq 0$. a, b, c and the constant were evaluated by regression analysis. The level of significance of each term in the equations was examined by the t test. Unless otherwise noted, all of the correlation equations and all of the terms in each equation were justified above the 95% level.

3 RESULTS

3.1 Evaluation of the tail-current activity

We measured the effects of compound **3** on the membrane current of the crayfish giant axon with the voltage-clamp technique. Before application of the compound, the inward current in response to a -20 mV test pulse increased rapidly and then decreased to give a peak current (Fig. 1A, arrow 1). By treating with $1{\cdot}0 \times 10^{-4}$ M compound **3** for 137 min, the amplitude of the peak current was reduced to about 85% of the control and the falling phase was slowed to induce a large residual current during the depolarization of the membrane (Fig. 1B, arrow 2). Upon step repolarization, a large and slowly decaying tail-current was induced (Fig. 1B, arrow 3). It took more than 50 ms until the tail current decayed to the zero level. Similar results were obtained with other compounds, although the rate of

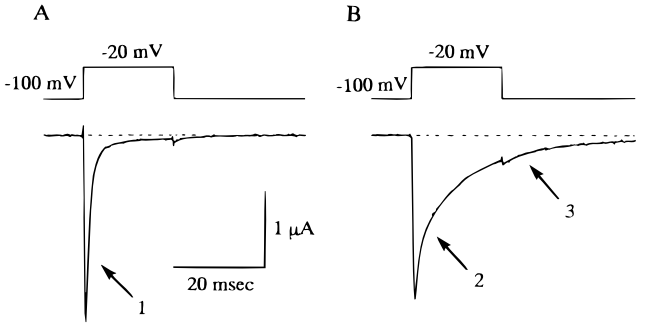


Fig. 1. Sodium currents recorded from the internally perfused giant axon. Axonal membrane was step-depolarized from holding potential (−100 mV) to −20 mV for 20 ms (A) before and (B) after 137 min of the internal application of compound **3** at the concentration of $1{\cdot}0 \times 10^{-4}$ M. The dotted lines indicate the zero level of the current. See text for the explanation of arrows 1–3.

decay of the tail-current was dependent on the compound. To quantify the rate of decay, the tail-current was redrawn with a semilog scale (Fig. 2). The semilog plots appear to give a straight line, so we considered that the falling phase of the tail-current obeys the first-order kinetics as shown in eqn (3):

$$\ln(-I_{tail}) = -t/\tau + \text{constant} \quad (3)$$

where $-I_{tail}$ ($I_{tail} < 0$) is the inward tail current (in μA), t is the time (in ms) after the step repolarization of the membrane and τ is the reciprocal of the rate constant of the decay.[9,14] The τ value for each compound was calculated by the least squares with eqn (3).

The log τ value of pyrethroid insecticides, which are a representative class of compounds which induce the tail-current, is not affected so much as to change the quality of the structure–activity relationships by experimental conditions, such as the length and potential of a test pulse and the concentration of compounds.[9,15,16] In fact, we confirmed for compound **3** that the log τ value is not much changed by the length and potential of a test pulse (Table 2). The activity values of compound **8** were not much different at $3{\cdot}3 \times 10^{-5}$ M (log $\tau = 1{\cdot}06$) and $5{\cdot}5 \times 10^{-5}$ M (log $\tau = 1{\cdot}05$), where the solutions were slightly turbid. Tail-currents induced by

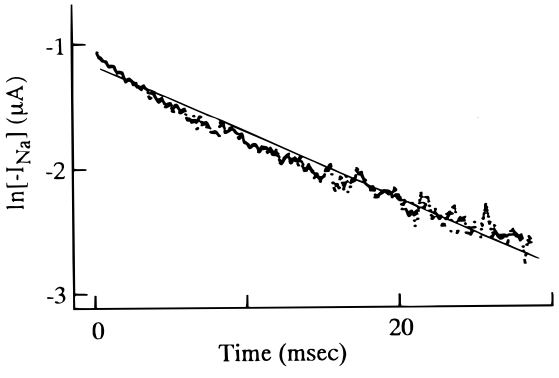


Fig. 2. Semilog plots of the tail-current against time. Original data were taken from Fig. 1B.

TABLE 2
Effects of Pulse Duration and Potential on Log τ Value of Compound **3**

Pulse duration (*ms*)	*Potential*	
	−20 mV	*20 mV*
10	1·31	1·32
15	1·34	1·33
20	1·37	1·34

this series of compounds were generally small even at saturated conditions as exemplified in Fig. 1 for compound **3**, so it was difficult to examine further the dependence of log τ on concentration. We considered that the dependence of the present series of compounds on the concentration is small, based on our previous findings for silaneophanes, which have generally low solubility in saline.[9] The larger the initial size of the tail-current, however, the more accurately the value of τ could be estimated. Thus, we selected appropriate experimental conditions to induce a large tail-current. Concentrations of compounds were in a range of $3{\cdot}3 \times 10^{-5}$ and $1{\cdot}2 \times 10^{-4}$ M. The size of the tail-current increases with time until a steady state is attained.[17] Therefore, the tail-current was measured at the steady state to calculate the τ value. The log τ values listed in Table 1 are the mean of at least two values estimated with depolarizing pulses of −20 and 20 mV. Two or more axonal preparations were used for some compounds, but a single axon was used for most of compounds as indicated in Table 1. The standard error of the mean value was less than $\pm 0{\cdot}2$.

3.2 Quantitative analysis of substituent effects on the tail-current activity

Among DCC-series compounds **1**–**17**, the log τ value varied from the most potent compound **9** ($X = OC_2H_5$) to the weakest compound **7** ($X = C(CH_3)_3$). Introduction of substituents to benzene rings increased the log τ value, except for the *tert*-butyl group. Variations in the log τ value of compounds **1**–**17** were analysed by use of physicochemical parameters of aromatic substituents with eqn (1) to give eqn (4) as the best one.

$$\log \tau = 0.248(\pm 0.280) + 0.843(\pm 0.275)\Delta L - 0.138(\pm 0.075)(\Delta B_5)^2 - 0.356(\pm 0.109)\pi^2 \quad (4)$$
$$n = 17,\ s = 0.211,\ r = 0.937,\ F_{3,13} = 31.31$$

In this and following equations, n is the number of compounds, s is the standard deviation, r is the correlation coefficient and F is the ratio between regression and residual variances. The figures in parentheses after each coefficient are the 95% confidence intervals of the

TABLE 3
Development of QSAR eqn (6)

Intercept	ΔL	$(\Delta B_5)^2$	π^2	I_P	I_T	s	r	$F(X, Y)^a$
0·714	0·248					0·580	0·399	4·54
0·122	1·038	−0·232				0·476	0·676	12·66
0·208	1·073	−0·202	−0·406			0·269	0·913	49·96
0·203	1·001	−0·182	−0·381	0·426		0·234	0·938	8·11
0·199	0·933	−0·164	−0·357	0·323	0·500	0·213	0·952	5·33

[a] F statistic for the significance of the addition of variables. X: The number of independent variables added at each step of the development. Y: *n-m*-l, *n* being the number of datum points and *m* being the total number of independent variables in the developed equation. Theoretical F values: $F(1, 24, 0{\cdot}05) = 4{\cdot}26$, $F(1, 23, 0{\cdot}05) = 4{\cdot}28$, $F(1, 22, 0{\cdot}05) = 4{\cdot}30$, $F(1, 21, 0{\cdot}05) = 4{\cdot}32$, $F(1, 20, 0{\cdot}05) = 4{\cdot}35$.

regression coefficient. Addition of ΔB_5 and/or π term(s) did not improve the correlation.

Even though the tail-current activity of DDT-, DDD- and prolan-series compounds was higher than for the corresponding compounds of the DCC-series except for compound **21** (Table 1), eqn (5) was obtained by including compounds **18**–**26**.

$$\log \tau = 0.208(\pm 0.324) + 1.073(\pm 0.284)\Delta L - 0.202(\pm 0.077)(\Delta B_5)^2 - 0.406(\pm 0.119)\pi^2 \quad (5)$$
$$n = 26,\ s = 0.269,\ r = 0.913,\ F_{3,22} = 36.89$$

For this analysis, substituent parameters for compounds **18**–**26** were taken from those of corresponding compounds **3**, **8** and **9** of the DCC-series. The results showed that the activities of DDT- and prolan-series compounds are slightly higher than others. Equation (6) was derived by use of two indicator variables for these two series of compounds, separately.

$$\log \tau = 0.199(\pm 0.258) + 0.933(\pm 0.239)\Delta L - 0.164(\pm 0.065)(\Delta B_5)^2 - 0.357(\pm 0.099)\pi^2 + 0.323(\pm 0.292)I_T + 0.500(\pm 0.292)I_P \quad (6)$$
$$n = 26,\ s = 0.213,\ r = 0.952,\ F_{5,20} = 38.35$$

In this and following equations, I_T is assigned to be unity for DDT-series compounds **18**–**20**, otherwise is zero. I_P is similarly assigned for prolan-series compounds **24**–**26**. Introduction of ΔB_5 and/or π term(s) did not improve the correlation. Equation (6) means that variations in the log τ value are parabolically related to the maximum width and hydrophobicity of the substituents, the optimum value being around zero for both parameters. The longer the substituent, the higher was the tail-current activity. Except for H of compound **1**, the ΔL and ΔB_5 values are positive as defined, so eqn (6) also means that introduction of a lengthy and narrow substituent is favourable to the tail-current activity. The I_T and I_P terms mean that the activity of DDT- and prolan-series compounds was two and three times, respectively, higher than others when other structural properties are the same. The development of eqn (6) and the intercorrelation of variables are shown in Tables 3 and 4, respectively. Log τ values calculated by eqn (6) are listed in Table 1.

3.3 Relationship between the insecticidal and tail-current activities

For 23 compounds that have both the definitive insecticidal and tail-current activity values, eqn (7) was obtained.

$$\log(1/\mathrm{MLD}) = 6.320(\pm 0.370) + 0.869(\pm 0.256)\log \tau \quad (7)$$
$$n = 23,\ s = 0.304,\ r = 0.839,\ F_{1,21} = 49.87$$

A close inspection of the results showed that the insecticidal activity calculated by eqn (7) for DDT-series compounds **18**–**20** is slightly higher than for others. By use of an indicator variable term I_T for the DDT-series compounds, eqn (7) was significantly improved to give eqn (8).

$$\log(1/\mathrm{MLD}) = 6.385(\pm 0.338) + 0.779(\pm 0.243)\log \tau - 0.437(\pm 0.372)I_T \quad (8)$$
$$n = 23,\ s = 0.274,\ r = 0.879,\ F_{2,20} = 33.88$$

This may be due to a specific structural feature of the DDT-series compounds. Furthermore, eqn (8) was

TABLE 4
Squared Correlation (r^2) Matrix for Variables Used to Derive eqn (6)

	ΔL	ΔB_5	$(\Delta B_5)^2$	π	π^2	I_T
ΔB_5	0·824					
$(\Delta B_5)^2$	0·843	0·920				
π	0·125	0·063	0·102			
π^2	0·116	0·119	0·127	0·166		
I_T	0·000	0·004	0·010	0·007	0·036	
I_P	0·000	0·004	0·010	0·007	0·036	0·017

improved to give eqn (9) by addition of the $(\log P)^2$ term.

$$\log(1/\mathrm{MLD}) = 6.767(\pm 0.421) + 0.764(\pm 0.214)\log \tau - 0.010(\pm 0.008)(\log P)^2 + 0.410(\pm 0.327)I_T \quad (9)$$
$$n = 23,\ s = 0.239,\ r = 0.914,\ F_{3,19} = 31.97$$

Addition of a log P term did not improve the equation. Equation (9) means that the insecticidal activity of these four series of compounds is basically governed by their tail-current activity and hydrophobicity; the higher the tail-current activity, the higher the insecticidal activity. Although the coefficient was small, the $(\log P)^2$ term was significant at a level higher than 95%. The insecticidal activity was parabolically related to the hydrophobicity, the optimum log P value being around zero. The log(1/MLD) values calculated by eqn (9) are listed in Table 1.

4 DISCUSSION

There are many examples in which variations in the biological activity of a set of compounds are parabolically correlated with log P.[18] These relationships can be explained by considering a compartment model.[19] Thus, a compound must pass through many hydrophobic and hydrophilic phases as barriers before reaching the site of action. Even in the sodium tail-current induced by a set of pyrethroid insecticides in the crayfish axonal membrane, the rate of development is parabolically related with log P.[17] We previously suggested that one of the barriers responsible for such results is axoplasmic material that remained around the internal surface of the axonal membrane, because test solutions were applied internally to the axon.[17] The hydrophobicity term in eqn (6) may reflect the significance of penetration of molecules through the axoplasmic material and/or into the axonal membrane. If so, the indicator variable term in eqn (6) would be involved in the hydrophobicity term when log P is used instead of π, because we cited the π values of DCC-series compounds **3**, **8** and **9** for those of other series of compounds **18**–**26**. However, such a trial gave a poorer correlation ($s = 0{\cdot}276$, $r = 0{\cdot}913$). These findings indicate that the hydrophobicity term in eqn (6) mostly reflects the hydrophobic interaction of the substituents with receptors such as sodium channels.

We previously analysed the relationship between the insecticidal activity against the American cockroach and the tail-current activity in the crayfish axonal membrane for various sets of pyrethroid insecticides.[9,15,16,20] In all cases, the log P term was significantly involved in the correlations. In some cases, further addition of a $(\log P)^2$ term improved the correlation with an optimum log P value in a range of 7·0–9·3.[9,16] We considered that the quadratic log P terms probably represent the hydrophobic factor participating in the transport process of compounds from the injected site in an insect body to the target. However, the optimum log P value in eqn (9) differed markedly from the reported values for pyrethroids. Thus, the hydrophobicity term in eqn (9) probably did not reflect simply the transport process of compounds to arrive at the target site for inducing the tail-current. If the term reflected the transport factor only, the optimum value should be close to the values calculated for pyrethroids, regardless of the set of compounds.

Previously, we quantitatively analysed variations in the activity of DCC-series compounds as used in this study to induce repetitive discharges in the central nerve cord of American cockroaches.[7] We found that variations in the activity in terms of log(1/MEC), where MEC is the minimum effective concentration to induce the repetitive discharges, are parabolically related to the steric parameter, the van der Waals volume. Recalculation by use of the STERIMOL parameter as used to derive eqn (6) gave eqn (10) as the best one.

$$\log(1/\mathrm{MEC}) = 4.693(\pm 0.384) + 1.749(\pm 0.554)\Delta B_5 - 0.308(\pm 0.187)(\Delta B_5)^2 - 0.745(\pm 0.501)\pi^2 \quad (10)$$
$$n = 16,\ s = 0.244,\ r = 0.928,\ F_{3,12} = 51.58$$

Equation (4) is quite similar to eqn (10), although the single steric terms are different from each other (ΔL was significant in eqn (4), ΔB_5 in eqn (10)). This might be partly due to high collinearity of these parameters ($r^2 = 0{\cdot}89$). Thus, we confirmed that the physicochemical properties of aromatic substituents of this series of compounds in affecting the potency at the axonal membrane level are quite similar to those at the nerve cord level, despite the differences in the nerve sources and in the application methods of compounds, as observed for pyrethroid insecticides of tetramethrin and related compounds.[21]

In summary, variations in the tail-current activity of DDT-type insecticides including the DCC-series compounds measured in crayfish giant axons were correlated with the STERIMOL length and width parameters as well as the hydrophobicity. The insecticidal activity of the compounds against the American cockroach was related with the tail-current activity when the hydrophobic factor was separately considered. The higher the tail-current activity, the higher was the insecticidal activity.

ACKNOWLEDGEMENTS

The authors thank Dr George Holan at CSIRO, Australia for providing us with some compounds and Mr

Hideki Masutani in Department of Agricultural Chemistry at Kyoto University for his technical assistance.

REFERENCES

1. Narahashi, T., Effects of insecticides on nervous conduction and synaptic transmission. In *Insecticide Biochemistry and Physiology*, ed. C. F. Wilkinson. Plenum, New York, 1976, pp. 327–52.
2. Lund, A. L. & Narahashi, T., Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroids and DDT analogs. *Pestic. Biochem. Physiol.*, **20** (1983) 203–16.
3. Corbett, J. R., Wright, K. & Baillie, A. C., Insecticides acting elsewhere in the nervous system. In *The Biochemical Mode of Action of Pesticides*. Academic Press, Essex, 1984, 2nd edn, pp. 141–78.
4. Vijverberg, H. P. M., van der Zalm, J. M. & van den Bercken, J., Similar mode of action of pyrethroids and DDT on sodium channel gating in myelinated nerves. *Nature* (*London*), **295** (1982) 601–3.
5. Lund, A. E. & Narahashi, T., Interaction of DDT with sodium channels in squid giant axon membranes. *Neuroscience*, **6** (1981) 2253–8.
6. Nishimura, K. & Fujita, T., Quantitative structure–activity relationships of DDT and its related compounds. *Nihon Noyaku Gakkaishi* (*J. Pestic. Sci.*), **8** (1983) 69–80.
7. Nishimura, K., Hirayama, K., Kobayashi, T., Fujita, T. & Holan, G., Quantitative structure–activity relationships of insecticidal diphenyldichlorocyclopropanes. *Pestic. Biochem. Physiol.*, **25** (1986) 153–62.
8. Lund, A. E. & Narahashi, T., Modification of sodium channel kinetics by the insecticide tetramethrin in crayfish giant axons. *Neurotoxicology*, **2** (1981) 213–29.
9. Okimoto, H., Nishimura, K., Matsuda, K., Hamada, M., Ueno, T. & Fujita, T., Neurophysiological effects and quantitative structure–activity analyses of insecticidal silaneophanes. *Pestic. Biochem. Physiol.*, **49** (1994) 83–93.
10. Hansch, C. & Fujita, T., ρ-σ-π analysis. A method for the correlation of biological activity and chemical structure. *J. Amer. Chem. Soc.*, **86** (1964) 1616–26.
11. Asao, M., Shimizu, R., Nakao, K., Nishioka, T. & Fujita, T., to be submitted to Japan Chemistry Program Exchange.
12. Verloop, A., The STERIMOL approach: Further development of the method and new applications. In *Pesticide Chemistry, Human Welfare and the Environment, Vol. 1*, ed. J. Miyamoto & P. C. Kearney. Pergamon, Oxford, 1983, pp. 339–44.
13. Fujita, T., Iwasa, J. & Hansch, C., A new substituent constant, π, derived from partition coefficients. *J. Amer. Chem. Soc.*, **86** (1964) 5175–80.
14. Lund, A. E. & Narahashi, T., Kinetics of sodium channel modification by the insecticide tetramethrin in squid axon membranes. *J. Pharmacol. Exp. Ther.*, **219** (1981) 464–73.
15. Kobayashi, T., Masutani, H., Nishimura, K. & Fujita, T., Quantitative structure–activity studies of pyrethroids. 16. Physicochemical and structural effects of tetramethrin and related compounds on the decay of sodium tail currents in crayfish giant axons. *Pestic. Biochem. Physiol.*, **35** (1989) 58–69.
16. Nishimura, K., Kobayashi, T. & Fujita, T., Quantitative structure–activity studies of pyrethroids. 23. Rate of decrease in sodium tail currents induced by substituted benzyl chrysanthemates and pyrethrates in crayfish giant axons. *Pestic. Biochem. Physiol.*, **40** (1991) 99–110.
17. Kobayashi, T., Nishimura, K. & Fujita, T., Quantitative structure–activity studies of pyrethroids. 13. Physicochemical properties and the rate of development of the residual and tail sodium currents in crayfish giant axon. *Pestic. Biochem. Physiol.*, **30** (1988) 251–61.
18. Lien, E. J., Structure–absorption–distribution relationships: significance for drug design. In *Drug Design, Vol. V*, ed. E. J. Ariëns. Academic Press, New York, 1975, pp. 81–132.
19. Penniston, J. T., Beckett, L., Bentley, D. L. & Hansch, C., Passive permeation of organic compounds through biological tissues: a non-steady-state theory. *Mol. Pharmacol.*, **5** (1969) 333–41.
20. Kobayashi, T., Nishimura, K. & Fujita, T., Effects of the α-cyano group in the benzyl alcohol moiety on insecticidal and neurophysiological activities of pyrethroid esters. *Pestic. Biochem. Physiol.*, **35** (1989) 231–43.
21. Kobayashi, T., Masutani, H., Nishimura, K. & Fujita, T., Quantitative structure–activity studies of pyrethroids. 16. Physicochemical and structural effects of tetramethrin and related compounds on the decay of sodium tail currents in crayfish giant axons. *Pestic. Biochem. Physiol.*, **35** (1989) 58–69.